

against a variety of Brucella species. The inventive protein is effective at eliciting immune response.

The protein described herein measures antibodies against proteins of the organism. Diagnostic probes based on the nucleotide sequence of the 28 kDa antigen can be made.

Finally, since the protein gives rise to immune response, the protein may be used as a subunit vaccine for brucellosis. Compositions containing the subject proteins or polypeptides of the protein in pharmaceutically acceptable carriers are useful as vaccines and as diagnostic agents to identify protective antibodies. The proteins may be attached to solid supports for use in diagnostic studies known in the art.

Both the 28 kDa protein and polypeptides containing over 90 amino acid peptides of the protein thereof, which are reactive with antibodies raised to brucella species, can be may be administered by mouth. Antigenic fusion proteins containing sequences of other proteins such as cholera subunits are useful for administration orally or to the mucosa (for example intranasally). The fusion proteins may be lyophilized and inhaled from a vial for administration.

Materials and Methods:

A genomic library of B. melitensis DNA was generated in lambda gt11 obtained from Promega Corp., Madison, Wisconsin. The bacterial genomic DNA was digested with HaeIII to generate fragments with an average size of 2.5 kilo base-pairs (kb). The DNA was then methylated with EcoRI methylase followed by addition of EcoRI linkers and digestion with EcoRI restriction enzyme.

sequence of interest shown below:

cccctgacataacccgctttgtccaaatTTTTTcaactTTTtctgtaggagattttatga
acaactcgtgctagcaatttttctcgcagcctcattttccacaatcatgctcgtcggcgctt
tcagcctgcccgcctttcgcacaggagaatcagatgacgacgcagcccgcgcgatcgccg
5 tcaccggggaaggcatgatgacggcctcgccgatatggccattctcaatctctcgggtgc
tacgccaggcaaagaccgcgcgcgaagccatgaccgcgaataatgaagccatgacaaaag
tgctcgtatgcatgaagaaggccggcatcgaagatcgcgatctccagacaggcggcatca
atatccagccgattttatgtctatcctgacgacaagaacaacctgaaagagcctaccatca
ccggctatttctgtatccaccagtctcacgggttcgcgtgcgcgaactggccaatggttgaa
10 aaatTTTtgatgaatccgtcacgctcgggtgtaatcagggcggtgatttgaacctggtca
atgataatccctccgccgtgatcaacgaggcgcgcaagcgcgagtgccaatgccattg
ccaaggcgaagacgcttgccgacgctgcaggcgtggggcttgccgctgtggtggaaatca
gtgaactgagccgcccgcgccatgccgatgccaattgcgcgcggacagttcagaacctatgc
tagcagccgcaccggacaattccgtgccgattgccgcaggcgaaaacagctataacgtat
15 cgggtcaatgtcgtTTTTtgaaatcaagtaaatagctgggggtatgacgccctttgccacctg
atacaaaacgcccggcctgggtttcacaggccgggtTTTTtgattagagcgcggtttcgatct
gattgaatccgatcggcgctctaatacctttgTTTTgacgcgcacatctttccgaaaaccgt
ttcacactTTTtcgggatgcggtctagcggatgatcgggcaaccgcgcgtatcggaatg
tcacg (Seq. ID No. 1)

20 A database search of the National Center for Biotechnology
Information database was performed. However, no significant
homologies were found. The gene encodes the 28 kDa antigen
contained within the genomic material of Brucella species. The
DNA/amino acid sequence was identified to be:

1
 cccctgacataacccgctttgtccaaatTTTTTcaacttttctgtaggagattttatga 60
 1 M N 20
 acactcgtgctagcaattttctcgcagcctcattttccacaatcatgctcgtcggcgctt 120
 T R A S N F L A A S F S T I M L V G A F 40
 tcagcctgcccgcttttcgcacaggagaatcagatgacgacgcagcccgcgcgatcgccg 180
 S L P A F A Q E N Q M T T Q P A R I A V 60
 tcaccggggaaggcatgatgacggcctcgcccgatatggccattctcaatctctcggtgc 240
 T G E G M M T A S P D M A I L N L S V L 80
 tacgccaggcaaagaccgcgcgcgaagccatgaccgcgaataatgaagccatgacaaaag 300
 R Q A K T A R E A M T A N N E A M T K V 100
 tgctcgatgccatgaagaaggccggcatcgaagatcgcgatctccagacaggcggcatca 360
 L D A M K K A G I E D R D L Q T G G I N 120
 atatccagccgatttatgtctatcctgacgacaagaacaacctgaaagagcctaccatca 420
 I Q P I Y V Y P D D K N N L K E P T I T 140
 ccggctattctgtatccaccagtctcacggttcgcggtgcgcgaactggccaatgttggaa 480
 G Y S V S T S L T V R V R E L A N V G K 160
 aaattttggatgaatccgtcacgctcggtgttaatcagggcgggtgatttgaacctggta 540
 I L D E S V T L G V N Q G G D L N L V N 180
 atgataatccctccgccgtgatcaacgaggcgcgcgaagcgcgcagtggccaatgccattg 600
 D N P S A V I N E A R K R A V A N A I A 200
 ccaaggcgaagacgcttgccgacgctgcaggcgtggggcttgccggtgtggtggaaatca 660
 K A K T L A D A A G V G L G R V V E I S 220
 gtgaactgagccgcccgcgccatgccgatgccaatcgcgcgcgacagttcagaacctatgc 720
 E L S R P P M P M P I A R G Q F R T M L 240
 tagcagccgcaccggacaattccgtgccgattgccgcaggcgaaaacagctataacgtat 780
 A A A P D N S V P I A A G E N S Y N V S 260
 cggtaaatgtcgtttttgaaatcaagtaaatactgggggtatgacgccctttgccacctg 840
 V N V V F E I K * 280
 atacaaaacgccggcctggttttcacaggccgggttttttgattagagcgcgttttcgatct 900
 gattgaatccgatcggcgctctaatacctttgttttgacgcgcacatcttttccgaaaaccgt 960
 ttcacacttttcgggatgcgggtctagcgggatgatcgggcaaccgcgcgtatcggcaaattg 1020
 tcacg 1025 (Seq. ID No. 1 (DNA) and Seq. ID No. 2 (protein))

Southern blots with a 28 kDa specific probe revealed that the gene is contained within a 8.5 kb EcoRI fragment in B. melitensis 16 M and Rev1, B. abortus 2308 and S19, B. suis, B. neotome, B. Canis and B. ovis.

The 28 kDa Brucella protein was shown to have the sequence:
Met Asn Thr Arg Ala Ser Asn Phe Leu Ala Ala Ser Phe Ser Thr Ile
Met Leu Val Gly Ala Phe Ser Leu Pro Ala Phe Ala Gln Glu Asn Gln
Met Thr Thr Gln Pro Ala Arg Ile Ala Val Thr Gly Glu Gly Met Met
Thr Ala Ser Pro Asp Met Ala Ile Leu Asn Leu Ser Val Leu Arg Gln
Ala Lys Thr Ala Arg Glu Ala Met Thr Ala Asn Asn Glu Ala Met Thr
Lys Val Leu Asp Ala Met Lys Lys Ala Gly Ile Glu Asp Arg Asp Leu
Gln Thr Gly Gly Ile Asn Ile Gln Pro Ile Tyr Val Tyr Pro Asp Asp
Lys Asn Asn Leu Lys Glu Pro Thr Ile Thr Gly Tyr Ser Val Ser Thr
Ser Leu Thr Val Arg Val Arg Glu Leu Ala Asn Val Gly Lys Ile Leu
Asp Glu Ser Val Thr Leu Gly Val Asn Gln Gly Gly Asp Leu Asn Leu
Val Asn Asp Asn Pro Ser Ala Val Ile Asn Glu Ala Arg Lys Arg Ala
Val Ala Asn Ala Ile Ala Lys Ala Lys Thr Leu Ala Asp Ala Ala Gly
Val Gly Leu Gly Arg Val Val Glu Ile Ser Glu Leu Ser Arg Pro Pro
Met Pro Met Pro Ile Ala Arg Gly Gln Phe Arg Thr Met Leu Ala Ala
Ala Pro Asp Asn Ser Val Pro Ile Ala Ala Gly Glu Asn Ser Tyr Asn
Val Ser Val Asn Val Val Phe Glu Ile Lys (Seq. ID No. 2)

The 28 kDa protein is obtained from lysates of the cell cultures and is purified by ion exchange to apparent homogeneity. Other methods known in the art appropriate for use include reverse phase HPLC and size exclusion chromatography.

Western blots of whole cell extracts of E. coli containing

the immunogenic peptides therefrom.

Immunogens are prepared by exposing the lysate to gel electrophoresis, then excising the 28 kDa bands from gel. The 28 kDa-containing band is then subjected to electrophoretic purification. The bands are pulverized in liquid nitrogen, then mixed with complete adjuvant and injected subcutaneously into the rabbits. The animals are given boosters after two weeks. Three to four days after the booster injection, the sera containing polyclonal antibodies is collected and screened. Serum containing antibodies can be detected by Western-blot.

Antibodies prepared against the 28 kDa antigen may be used to identify the infectious organisms in body fluids of mammals suspected of having brucellosis. Activity of monoclonal and polyclonal antibodies against 28 kDa antigens can be tested by several means. Western blot is used for the initial screening using 28 kDa bands as electrophoretically transferred to nitrocellulose paper. This screening procedure selects for high affinity antibodies, which are bound to the antigenic protein, since they must survive stringent washing methods. The monoclonal antibodies are used in screening the cDNA libraries. ELISA methodology can also be used as an alternate for initial screening by detecting binding of antigenic 28 kDa protein to antibodies.